Biohydrogenesis in the Thermotogales

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ABSTRACT: The bacterial order Thermotogales consists of obligate fermentative anaerobes, most that grow optimally in the range of 65-80°C. These bacteria are characterized by their unique "toga"-like outer membrane, which plays a role in breaking down a wide range of complex polysaccharides. Thermotogales are also capable of producing hydrogen with a high yield approximately twice that of mesophilic bacteria (3-4 mol H_2/mol glucose compared to 1-2). The objectives of this project are:

1. **Examine the regulation of substrate catabolic proteins and pathways as this relates to carbon partitioning, disposition of reducing power, and H_2 generation in *Thermotoga maritima* (*Tma*).**
2. **Dissect catabolic and regulatory pathways using genetic approaches based on past success with other hyperthermophiles.**
3. **Thermotogales biodiversity arises from adaptive specialization that expands on a conserved minimal genome; physiological characterization of selected novel traits will be done to expand understanding of biohydrogenesis.**

***Thermotoga* genetics.** During the past year our lab has focused on advancing targeted integration technology for *Thermotoga maritima* cell line construction combined with studies on the metabolism of this organism as it pertains to redox homeostasis. An additional promoter, *Msed_1271p* (pilin), has been developed to drive expression of *T. maritima pyrE* that provides expanded versatility to the *T. maritima* genetic system. qRTPCR quantitation of promoter strength has been conducted based on the transcript abundances of *groES*, *Msed_1271*, *hisI*, *ldh*, *rpoD* and transgene *kan* providing insight into transgenic cell lines. While other genetic markers remain in play, an emphasis has been placed on the use of natural and synthetic alleles of *pyrE*, therefore multiple cycles of *pyrE* transformation and recombination have been conducted to paramatize the process. Advances in studies on *T. maritima* metabolism include genetic screens for redox homeostasis mutants and new information on metal reduction that provide insights into regulation of hydrogen formation.

Metal reduction by *T. maritima*. We have discovered that *T. maritima* actively forms magnetic iron (magnetite) using insoluble synthetic ferrihydroxides in a process that is driven by sugar fermentation. Metal reduction is a preferred electron sink that shifts excretion of reductant from synthesis of hydrogen gas to formation of ferrous iron. Negative staining transmission electron microscopy demonstrates synthesis of pili during this process. Genes implicated in this process have been targeted for inactivation to test predictive models.

Phylogenetic analyses revealed likely *ompA* and *ompB* homologs among Thermotogales species. Since OmpA1 and OmpB are the two dominant structural proteins of the *T. maritima* toga and all known species of the Thermotogales have togas, we examined the genome sequences of other members of the Thermotogales to identify possible homologs of these proteins. The closest OmpB pBLAST sequence outside the Thermotogales was from *Vibrio mimicus* with an E value of 8×10^{-6} indicating that currently there are no closely related sequences outside the Thermotogales in the GenBank database. OmpB orthologs are present in *Thermosipho melanesiensis*, *Fervidobacterium nodosum*, and most species of the *Thermotoga* genus. However no OmpB homologs were found in *Thermotoga lettingae*, *Thermosipho africanus*, *Kosmotoga olearia, Petrotoga mobilis*, and Thermotogales bacterium mesG.Ag.4. The *ompB* orthologs lie in syntenic regions containing four genes: *secG-tyrS* (tyrosyl-tRNA synthetase)-*ompA1-ompB*. The organisms that lack homologs to *ompB* also have this syntenic region, but have a gene downstream of their *ompA1* homolog that encodes a protein with no homologs in the GenBank

nonredundant database (Fig. 3). The exception is that the *K. olearia* protein and the Thermotogales bacterium mesG.Ag.4 protein are homologous to one another, but to no other sequences. All these non-homologous protein sequences were examined for porin characteristics and all had many porin characteristics, except that they do not appear to be globular proteins. Though these proteins are not recognizable homologs of *T. maritima* OmpB, they may be analogs serving the same function.

Mesophilic member of the Thermotogales. A novel mesophilic member of the Thermotogales designated MesG1.Ag.4.2 was isolated from sediments from Baltimore Harbor, Maryland, USA. Cells of strain MesG1.Ag.4.2 were non-motile ovoids with sheath-like structures (togas). The strain was Gram-negative and grew optimally at 37ºC with a doubling time of 16.5 h (specific growth rate = 0.06 h^{-1}) on xylose at 37°C. It grew best on carbohydrates, but proteinaceous compounds also supported growth. This is the first describes species of the mesotoga lineage and provides new possibilities for the development of genetic methods for the Thermotogales.

Thermotoga species differ with respect to sugar utilization. Four hyperthermophilic members of the bacterial genus *Thermotoga* (*T. maritima, T. neapolitana, T. petrophila,* and *T.* sp. RQ2) share a core genome of 1470 open reading frames (ORFs), or about 75% of their genomes. Nonetheless, each species exhibited certain distinguishing features during growth on simple and complex carbohydrates that correlated with genomic inventories of specific ABC sugar transporters and glycoside hydrolases. These differences were consistent with transcriptomic analysis based on a multi-species cDNA microarray. Growth on a mixture of six pentoses and hexoses showed no significant utilization of galactose or mannose for any of the four species. *T. maritima* and *T. neapolitana* exhibited similar monosaccharide utilization profiles, with a strong preference for glucose and xylose over fructose and arabinose. *T.* sp. RQ2 also used glucose and xylose, but was the only species to utilize fructose to any extent, consistent with a phosphotransferase system (PTS) specific for this sugar encoded in its genome. *T. petrophila* used glucose to a significantly lesser extent than the other species. In fact, the XylR regulon was triggered by growth on glucose for *T. petrophila*, which was attributed to the absence of a glucose transporter (XylE2F2K2), otherwise present in the other Thermotoga species. This suggested that *T. petrophila* acquires glucose through the XylE1F1K1 transporter, which primarily serves to transport xylose in the other three Thermotoga species. The results here show that subtle differences exist among the hyperthermophilic Thermotogales that support their designation as separate species.

TM1300 locus in the *T. maritima* genome. The TM1300-1338 locus in the *T. maritima* genome contains a number of hypothetical proteins, including several that correspond to ORFs of less than 100 aa. Analysis of this locus has revealed that several putative bacteriocins and toxin-antitoxins (HicAB) are encoded, suggesting a key role in post-transcriptional regulation and possible anti-microbial actions. The gene encoding TM1312 has been expressed and confirmed to be a toxin (ribonucleases). Purification of TM1316, a putative cyclic peptide and suspected bacteriocin, is underway. Also, gel shift assays have been used to investigate two transcriptional regulators in this locus. For example, TM1330 appears to be involved in regulation of the region upstream of TM1316.

Publications in the past year citing DOE GTL Support:

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